

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/705, 16/28, G01N 33/68		A1	(11) International Publication Number: WO 99/07847 (43) International Publication Date: 18 February 1999 (18.02.99)
(21) International Application Number:	PCT/US98/14944	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date:	20 July 1998 (20.07.98)		
(30) Priority Data: 08/906,365	5 August 1997 (05.08.97)	US	
(71) Applicant: AMERICAN HOME PRODUCTS CORPORATION [US/US]; Five Giralda Farms, Madison, NJ 07940 (US).			
(72) Inventors: BHAT, Ramesh, A.; 738 Champlain Drive, King of Prussia, PA 19406 (US). HENDERSON, Ruth, Ann; 1414 Conway Drive, Swarthmore, PA 19081 (US). HSIAO, Chulai; 1907 Harwyn Road, Wilmington, DE 19810 (US). KARATHANASIS, Sotirios, Konstantinou; 862 Old State Road, Berwyn, PA 19312-1443 (US).			Published <i>With international search report.</i>
(74) Agents: WALSH, Andrea, C.; American Home Products Corporation, Patent Law Dept.-2B, One Campus Drive, Parsippany, NJ 07054 (US) et al.			

(54) Title: HUMAN ESTROGEN RECEPTOR-BETA

(57) Abstract

The present invention provides isolated nucleic acids encoding full-length human estrogen receptor- β (hER β), which comprises 530 amino acids. The invention also provides isolated hER β polypeptides and hER β -reactive antibodies, including those that specifically recognize amino acids 1-45 of hER β . The invention also encompasses methods for identifying hER β -interactive compounds, including agonists, antagonists, and co-activators.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

HUMAN ESTROGEN RECEPTOR-BETA

Field of the Invention

This invention pertains to DNA encoding a novel human estrogen receptor- β (hER β), hER β polypeptides, and methods for expressing and isolating hER β . The invention also pertains to methods for using hER β to identify coactivators and inhibitors as well as tissue-specific estrogens.

Background of the Invention

10 The physiological response to steroid hormones is mediated by specific interactions of steroids with nuclear receptors, which are ligand-activated transcription factors that regulate the expression of target genes by binding to specific DNA response elements. These receptors comprise (in an aminoterminal-to-carboxyterminal direction) a hypervariable
15 aminoterminal domain that contributes to the transactivation function; a highly conserved DNA-binding domain responsible for receptor dimerization and specific DNA binding; and a carboxyterminal domain involved in ligand-binding, nuclear localization, and ligand-dependent transactivation.

Recently, cDNA was cloned from rat prostate and was shown to
20 have significant homology to a previously isolated rat estrogen receptor cDNA. Kuiper et al., Proc.Natl.Acad.Sci.USA 93:5925, 1996. This receptor was designated ER β to distinguish it from a previously cloned receptor, ER α . Rat ER β was shown to be expressed in the prostate, testes, ovary, and thymus, in contrast to ER α , which is most highly expressed in
25 the uterus, breast, liver, and pituitary.

A human ER β homologue having the aminoterminal sequence Gly-Tyr-Ser has been reported. Mosselman et al., FEBS Letts. 392:49, 1996. This reported sequence lacks an initiator methionine, however; therefore, the complete aminoterminal sequence could not be determined. Thus, the
30 full-length human gene remained unknown and an accurate picture of the

molecular determinants of the transactivation function of authentic hER β could not be obtained.

Summary of the Invention

5 The present invention provides nucleic acids encoding a full-length human estrogen receptor- β (hER β L). The nucleic acid sequence of hER β L, which is depicted in Figure 3, SEQ ID NO:1, encodes a receptor having the amino acid sequence depicted in Figure 4, SEQ ID NO:2. hER β L according to the present invention contains 45 amino acids at its aminoterminal
10 10 which were not previously known. These amino acids are believed to contribute to the transcription activation function of the receptor.

15 hER β L is selectively expressed in the thymus, spleen, ovary, and testes. Accordingly, hER β can be used to identify co-activator proteins that are involved in estrogen-regulated gene expression, as well to identify tissue-selective estrogens.

20 The present invention provides isolated polypeptides having the sequence of SEQ ID NO:2 and function-conservative variants thereof which exhibit estrogen-regulated transcriptional activation activity. In a related aspect, the invention encompasses isolated peptides derived from hER β comprising a sequence corresponding to amino acids 1-45 of SEQ ID NO:2 and function-conservative variants thereof, as explained above. It is believed that this sequence provides at least part of the transactivation function.

25 The present invention also provides isolated nucleic acids encoding hER β L and hER β L-derived peptides, including the nucleic acid sequence depicted in Figure 3, SEQ ID NO:1 and subfragments thereof encoding peptides which comprise amino acids 1-45, as well as sequence-conservative and function-conservative variants thereof. Also encompassed by the invention are DNA vectors comprising an hER β L-encoding sequence operably linked to a transcription regulatory element and cells comprising these vectors. Methods for producing hER β L-derived

polypeptides include incubating a cell comprising an hER β L-encoding expression vector under conditions that permit expression of one or more hER β polypeptides. The methods further include: (a) harvesting the cells to produce a cell fraction and a medium fraction; and (b) recovering the 5 polypeptide(s) from the cell fraction, medium fraction, or both.

In another aspect, the invention provides methods for identifying hER β -interactive compounds, including agonists, antagonists, and co-activator proteins. In one embodiment, the method includes:

- (a) contacting purified hER β with a labeled ligand in the 10 presence of test compounds, to form test reactions, and in the absence of test compounds, to form control reactions;
- (b) incubating the test and control reactions under appropriate conditions to achieve specific binding of the labelled ligand to hER β ;
- 15 (c) determining the level of binding of the labeled ligand to hER β in said test and control cultures; and
- (d) identifying as a hER β -interactive compound any compound that reduces the binding of the labeled ligand to hER β .

20 **Brief Description of the Drawings**

Figure 1 is a schematic illustration of the oligonucleotides used for PCR amplification of human estrogen receptor- β (hER β L) cDNA (SEQ ID NO:5 and SEQ ID NO: 6).

Figure 2 is a schematic illustration of the pcDNA3 plasmid 25 containing hER β L cDNA.

Figure 3 is an illustration of the full-length cDNA sequence encoding human estrogen receptor- β (hER β L) (SEQ ID NO:1).

Figure 4 is an illustration of the predicted amino acid sequence of 30 the hER β polypeptide (SEQ ID NO:2). The first 45 (previously unknown) amino acids are underlined.

Figure 5 is a photographic illustration of an autoradiogram of a 10% SDS-polyacrylamide gel in which hER β in vitro translation products are resolved. Lane 1, hER β T; lane 2, hER β L produced from a vector encoding a synthetic translation initiation site; lane 3, hER β L produced from a vector 5 encoding the natural hER β translation initiation sequences.

Figure 6A is a graphic illustration of the transcriptional activation capacity of full-length hER β (hER β L) and truncated hER β (hER β T) expressed in HepG2 cells. Cells were transfected with either hER β L or hER β T and co-transfected with a luciferase reporter plasmid containing an estrogen 10 response element (ERE) (ERE.TK.LUC) and a control β -galactosidase plasmid. Cells were incubated in the absence or presence of estradiol, after which luciferase activity was measured and normalized to β -galactosidase activity.

Figure 6B is a graphic illustration of luciferase activity in HepG2 cells 15 transfected with either hER β L or hER β T and co-transfected with a luciferase reporter plasmid lacking an ERE (TK.LUC)

Figure 7A is a graphic illustration of the effect of estradiol stimulation of full-length hER β (hER β L) and truncated hER β (hER β T) on NF κ B activation in HepG2 cells. Cells were transfected with either hER β L or 20 hER β T and co-transfected with a luciferase reporter plasmid containing three copies of an NF κ B binding site (3X-NF κ B TK.LUC) and a control β -galactosidase plasmid. Cells were stimulated with interleukin-1 β and incubated in the absence or presence of estradiol, after which luciferase activity was measured and normalized to β -galactosidase activity. Figure 25 7B is a graphic illustration of luciferase activity in cells transfected with either hER β L or hER β T and co-transfected with a luciferase reporter plasmid lacking an NF κ B binding site (TK.LUC).

Figure 8 is a graphic illustration of the transcriptional activation capacity of full-length hER β (hER β L) and truncated hER β (hER β T) expressed 30 in HAECT-1 human endothelial cells. Cells were transfected with either hER β L or hER β T and co-transfected with a luciferase reporter plasmid

containing an ERE (ERE.TK.LUC) or one lacking an ERE (TK.LUC). Cells were incubated in the absence or presence of estradiol, after which luciferase activity was measured. ERE TK.LUC values were normalized to TK.LUC values and are presented as mean \pm S.E. (n=4).

5 Figure 9 is a graphic illustration of the effect of increasing doses of estrogens (17- β estradiol or genistein) on the transcriptional activation capacity of full-length hER β (hER β L) and truncated hER β (hER β T) expressed in *S. cerevisiae*. Cells were transformed with either hER β L or hER β T and co-transformed with a β -galactosidase reporter plasmid containing an ERE.

10 Transformed cells were treated with estrogens for 3h and assayed for β -galactosidase activity.

Detailed Description of the Invention

Human estrogen receptor- β (hereinafter, hER β) comprises
15 aminoterminal amino acid residues not previously known. The present invention encompasses isolated, purified, nucleic acids encoding authentic full-length hER β of 530 amino acid residues and fragments thereof which include nucleic acids encoding amino acids 1-45 of hER β . The invention also encompasses isolated, purified, polypeptides comprising hER β and
20 peptides derived therefrom, particularly peptides which include residues 1-45 of hER β . The invention also provides expression systems in which transcriptionally active hER β or fragments derived therefrom can be produced, as well as screening methods for identifying hER β agonists and antagonists (including tissue-specific estrogens and anti-estrogens) as well
25 as hER β co-activators and inhibitors.

Isolation and Characterization of the Gene Encoding hER β

The present inventors have isolated the cDNA encoding hER β using the methods outlined below. Human testis Poly A + RNA (1 mg, Clontech,
30 Palo Alto CA) was mixed with 0.5 mg oligo dT primer (GIBCO-BRL, Gaithersburg MD) in a total volume of 10 ml. The mixture was heated at

70°C for 10 minutes, and, after cooling on ice, was supplemented with 500 mM of each deoxynucleoside triphosphate, 1X cDNA synthesis buffer, and 10 mM DTT to a final reaction volume of 20 ml. The mixture was incubated at 42°C for 2.5 minutes and then supplemented with 1-2 units 5 reverse transcriptase (GIBCO-BRL, Gaithersburg MD), after which it was incubated at 45°C for 30 minutes and 50°C for 5 minutes. One-tenth of this mixture (approximately 2 ml) containing the cDNA template was then used in PCR amplification of hER β using forward and reverse primers as described below.

10 Alignment of the known rat ER β sequence (Kuiper et al., Proc.Natl.Acad.Sci.USA 93:5925, 1996) with that of a human homologue (Mosselman et al., FEBS Letts. 392:49, 1996) suggested that the human sequence lacked at least the ultimate and penultimate residues at its aminoterminus, as shown below:

15 Rat: MTFYSPAVMNYS . . . (SEQ ID NO:3)
Human: - - GYSPAVMNYS . . . (SEQ ID NO:4)

Based on this information, PCR primers were designed that supplement the human sequence with the two missing aminoterminal residues M and T and with an artificial Kozak translation initiation sequence. The forward primer, 20 having the sequence (SEQ ID NO: 5)
5'-GGAAGCTTGTGACCATCATGACCGGCTATGCCCTGCTGTGATG-3'
and a reverse primer, having the sequence (SEQ ID NO:6)
5'-GGATCTAGAGTCGACGCGTCACTGAGACTGAGGGTTCTGG-3'
were used to amplify hER β sequences in a reaction containing the following 25 components: 2 ml of the cDNA template described above; 1X PCR buffer; 200 mM of each deoxynucleoside triphosphate, 2 units of hot tab polymerase (Amersham, Arlington Heights IL), and 1mg of each of the forward and reverse primers. The reaction mixture was heated to 95°C for 2 minutes, annealed at 52°C for 1 minute, and amplified using 36 cycles of 30 72°C for 1.5 minutes.

A fragment of approximately 1500 bp in length was produced. The fragment digested with HindIII and XbaI (which cleave at sites present in the forward and reverse primer sequences, respectively, but not in the main body of the amplified cDNA sequence) and cloned into the corresponding sites of the pcDNA3 expression vector (Invitrogen, Carlsbad CA). This asymmetric cloning strategy places the 5' end of hER β cDNA under the control of the viral CMV promoter in pcDNA3 (Figures 1 and 2). Several insert-containing pcDNA3 clones were identified. Plasmid DNA was prepared from three clones using a plasmid purification kit (Qiagen, Santa Clarita CA) and their insert sequences were determined by the dideoxy termination method. One clone (designated R61010-2.24 or Clone 3) was found to contain an insert with a nucleotide sequence identical to the published hER β sequence (Mosseman et al., FEBS Letts. 392:49, 1996) and had the following 5' end structure:

15 M T G Y . . . (SEQ ID NO:7)
 CCATC ATG ACC GGC TAT . . . (SEQ ID NO:8)

This clone was designated "truncated hER β " or hER β T.

To verify the aminoterminal and upstream sequence of human hER β , two independent approaches were taken, as described below.

20 (1) 10 ml of a human ovary 5'-Stretch cDNA library (Clontech, Palo Alto CA) was mixed with 50 ml of 1X K solution (1X PCR Buffer (GIBCO-BRL, Gaithersburg MD), 2.5 mM MgCl₂, 0.5% Tween-20, 100 mg/ml Proteinase K), and the reaction mixture was incubated at 56°C for 2 hours, then at 99°C for 10 minutes. 5 ml of this reaction mixture 25 were then used as template in a nested PCR reation. For the first round, the forward primer (pDR2 sequencing primer, Clontech, Palo Alto CA) had the sequence 5'-CTGGTAAGTTAGTCTTTTGTC-3' (SEQ ID NO:9), and the reverse primer (hER β -specific, designated oligo #12908) had the sequence 5'-GCTTCACACCAAGGACTCTTTGAG-3' (SEQ ID NO:10).

30 The reaction contained 1X Klentaq PCR reaction buffer (40 mM Tricine-KOH, 15 mM KOAc, 3.5 mM Mg(OAc)₂, 75 mg/ml bovine serum albumin);

0.2 mM of each dNTP; 0.2 mM of each of the above primers, and 1 unit of Klentaq Polymerase Mix (Clontech, Palo Alto CA). Touchdown PCR conditions were as follows: 5 cycles of 94°C for 2 seconds and 72°C for 4 minutes, followed by 30 cycles of 94°C for 2 seconds and 67°C for 3

5 minutes.

Excess nucleotides and primers were removed from first round PCR reactions by purification over Wizard PCR columns (Promega, Madison WI). A second-round PCR reaction was then performed using 2 ml of the purified first-round reaction mixture. For the second round, the forward primer was
10 the pDR2 sequencing primer shown above, and the reverse primer had the sequence 5'-GTTGGCCACAACACATTGGGCTTGT-3' (hER β -specific, designated oligo #13871) (SEQ ID NO:11). The PCR reaction and cycling conditions were identical to those employed in the first round. The products were cloned into pCR2.1 (Invitrogen) and three resulting clones
15 were sequenced. All three clones (designated L1, L2, and L3) contained hER β inserts of different lengths, all of which were homologous to hER β and to each other.

(2) A Marathon Ready thymus cDNA kit (Clontech) for 5' rapid amplification of cDNA ends (RACE) was also used to isolated hER β 5' clones. In the first round of a nested PCR reaction, 5 ml of human thymus Marathon-ready cDNA (Clontech) was used as template. The forward primer had the sequence 5'-CCATCCTAATACGACTCACTATAAGGC-3' (Adaptor primer 1, Clontech) (SEQ ID NO:12), and the reverse primer had the sequence 5'-GCTTCACACCAAGGACTCTTGAG-3' (hER β -specific, designated oligo #12908) (SEQ ID NO:10). The PCR reaction and cycling conditions were identical to those described in (1) above.

Excess nucleotides and primers were removed from the first round PCR reactions by purification over Wizard PCR columns (Promega). A second round PCR reaction was performed using 2 ml of the purified first
30 round reaction. For the second round, the forward primer had the sequence 5'-ACTCACTATAAGGCTCGAGCGGC-3' (nested adaptor primer 2,

Clontech) (SEQ ID NO:13), and the reverse primer had the sequence 5'-GTTGGCCACAAACACATTGGGCTTGT-3' (hER β -specific, designated oligo #13871) (SEQ ID NO:11). The second round PCR reaction and cycling conditions were identical to those employed in the first round. The 5 products were cloned into the pCR2.1 vector and two clones were sequenced. The two clones contain insert sequences of different lengths that are homologous to hER β , to each other, and to the sequences isolated from a human ovary cDNA library as described above.

All of the hER β sequences isolated by methods (1) and (2) above 10 contained 110 nucleotides corresponding to hER β T sequences, as well as 228 additional nucleotides at the 5' end (Figure 3).

The hER β cDNA sequence determined from these clones contained several important differences from the previously known human sequence. First, the third amino acid of the previous sequence was found to be F and 15 not G (see above). Second, the methionine residue at the aminotermminus of the previous sequence was found not to be the initiator (i.e., true aminoterminal) residue. Rather, the authentic full-length hER β cDNA sequence encodes a polypeptide having 530 residues, the first 45 of which are not found in the previously known human sequence (Figure 4). The 20 sequence appears to be quite homologous to rat ER β ; however, this reading frame was not identified previously (Kuiper et al., Proc.Natl.Acad.Sci.USA 93:5925, 1996). Furthermore, an optimal Kozak translation initiation sequence is found upstream of the newly discovered initiator methionine codon. A termination codon was identified 63 nucleotides upstream to the 25 authentic ATG initiator codon in the same reading frame.

The cDNA encoding authentic full-length hER β was cloned into pCDNA3 under the control of the CMV promoter; this expression vector was designated "long hER β " or hER β L.

Synthesis of full-length hER β and truncated hER β

To examine the natural start site for translation of hER β , three plasmids were subjected to coupled transcription-translation, encoding hER β T (with a synthetic upstream translation initiation sequence), hER β L (with a synthetic upstream translation initiation sequence), and hER β L containing 93 nucleotides of its native upstream sequence (the entire sequence shown in Figure 3). The plasmids were transcribed and translated using the TNT T7 Coupled Reticulocyte Lysate System (Promega #L4610). Circular plasmid DNA was purified using Qiagen Maxi-Kit #12362. 2mg of the DNA was transcribed and translated in a single reaction in the presence of [35S]-methionine (New England Nuclear, Boston MA). The translation products were resolved on a 10% SDS polyacrylamide gel and were visualized by autoradiography (Figure 5).

The resulting translation products of both hER β L products were of similar size (~63 kDa), and the hER β T product was appropriately shorter (~56 KDa). This indicates that the initiator ATG most likely utilized in vivo is the ATG at position 94-96. Utilization of a further upstream ATG is unlikely because of a termination codon in-frame with the presumed start site. Confirmation of the authentic start site is achieved by subjecting hER β polypeptides to aminoterminal sequencing.

Functional differences between full-length hER β and truncated hER β

The experiments described below were performed to evaluate the transcription activation properties of full-length hER β according to the present invention and to compare it with that of truncated hER β . hER β L and hER β T were expressed in parallel in different cell types and tested for their ability to transactivate reporter genes containing estrogen response elements (EREs). Alternatively, hER β L and hER β T may be expressed in host cells containing endogenous estrogen-responsive genes and the estrogen-mediated activation of the endogenous genes is measured.

(i) HepG2 Cells:

HepG2 cells (ATCC) were transfected in parallel with either pcDNA3-hER β L or pcDNA3-hER β T using the calcium phosphate co-precipitation method. Cells were co-transfected with a reporter plasmid containing a luciferase gene preceded by either an ERE upstream of the thymidine kinase (TK) basal promoter, or the TK basal promoter alone. Cells also received a plasmid encoding β -galactosidase under the control of an RSV promoter, which was used to correct for variation in DNA uptake. Five hours after transfection, cells were incubated with or without 10-6M 17- β estradiol for 20 hours, after which cell extracts were prepared. Luciferase activity was measured by a chemiluminescent method using the Promega luciferase assay system, and β -galactosidase activity was measured by Galactolight (Tropix, Inc., Bedford MA); luciferase activity was then normalized to β -galactosidase activity.

The results shown in Figure 6A indicate that, in the presence of estradiol, hER β T caused a 2-fold stimulation of ERE activity. By contrast, hER β L under the same conditions caused a 6-fold stimulation of ERE activity. Thus, hER β L is about 3-fold more active than hER β T in this circumstance.

In a separate experiment, HepG2 cells were transfected with hER β L or hER β T as above, but the reporter gene consisted of three copies of an NF κ B binding site upstream of the TK basal promoter. Transfected cells were incubated with or without interleukin-1b (IL-1b) to activate NF κ B and/or with estradiol prior to luciferase determination. The results shown in Figure 7 indicate that hER β L was capable of attenuating the IL-1b-mediated NF κ B transcriptional activation (to an extent similar to that observed with hER α), while hER β T exhibited no inhibitory activity.

(ii) Human endothelial cells:

HAECT-1 cells (a clonal immortalized human aortic endothelia cell line derived by infection with Ad5 ori-SV40 ts A209) were transfected with pcDNA3hER β T or pcDNA3-hER β L and ERE-luciferase plasmids by

electroporation. After 4 hours, the cells were treated overnight with or without 100 nM 17- β estradiol prior to luciferase activity measurements. The results shown in Figure 8 indicate that hER β L is 2-3 times more active than hER β T in activating the ERE-reporter gene in the presence of estradiol.

5 In independent experiments, cells transfected under identical conditions were monitored for their levels of estrogen receptors using a ligand binding assay. The results indicate that the increased activity of hER β L relative to hER β T is not due to an increase in receptor number or stability, and, further, that the 2-3 fold increment measured in the above experiment may be an
10 underestimate of the true transactivational capacity of hER β .

(iii) Yeast:

S. cerevisiae strain BJ2168 (Yeast Genetic Stock Center, Berkeley CA) was co-transformed with an ERE-LacZ reporter plasmid (designated YRpE2) and yeast vectors expressing either hER β L or hER β T under the control of the yeast triose phosphate isomerase promoter in the yeast pYX242 vector (R&D Systems, Minneapolis MN). Transformed cells were grown in selective medium for 24 hours, after which they were treated in the presence or absence of increasing concentrations of either 17- β estradiol or the phytoestrogen Genistein (Research Biochemical International, Natick MA) for 3 hours prior to determination of β -galactosidase activity. The dose-response results shown in Figure 9 indicate that the maximal level of estrogen-stimulated LacZ expression was 2-fold higher in hER β L-transformed cells relative to hER β T-transformed cells.

25 DNA, Vectors, and Expression Systems

Many conventional techniques in molecular biology, microbiology, and recombinant DNA, are used in practicing the present invention. See, for example, Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; DNA Cloning: A Practical Approach, Volumes I and II, 30 1985 (D.N. Glover ed.); Oligonucleotide Synthesis, 1984, (M.L. Gait ed.);

Nucleic Acid Hybridization, 1985, (Hames and Higgins); Transcription and Translation, 1984 (Hames and Higgins eds.); Animal Cell Culture, 1986 (R.I. Freshney ed.); Immobilized Cells and Enzymes, 1986 (IRL Press); Perbal, 1984, A Practical Guide to Molecular Cloning; the series, Methods in 5 Enzymology (Academic Press, Inc.); Gene Transfer Vectors for Mammalian Cells, 1987 (J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory); and Methods in Enzymology Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively).

The present invention encompasses purified, isolated, nucleic acid 10 sequences encoding hER β , including, e.g., the nucleotide sequence depicted in Figure 3 SEQ ID NO:1 and subfragments derived therefrom, including without limitation transcriptional activation-competent fragments. An "isolated" or "purified" nucleic acid is a nucleic acid or polypeptide that is removed from its original environment (for example, its natural environment 15 if it is naturally occurring). An isolated nucleic acid or polypeptide contains less than about 50%, preferably less than about 75%, and most preferably less than about 90%, of the cellular components with which it was originally associated.

A nucleic acid that is "derived from" an hER β sequence is a nucleic 20 acid sequence that corresponds to a region of the sequence, sequences that are homologous or complementary to the sequence, and "sequence-conservative variants" and "function-conservative variants". Sequence-conservative variants are those in which a change of one or more nucleotides in a given codon position results in no alteration in the amino 25 acid encoded at that position. Function-conservative variants are those in which the amino acid sequence of hER β has been changed without altering the overall conformation and transcriptional activation function of the hER β polypeptide, including, but not limited to, replacement of an amino acid with one having similar physico-chemical properties (such as, for example, 30 acidic, basic, hydrophobic, and the like). A large number of candidate function-conservative hER β variants, as well as fragments of hER β that

retain transcriptional activation activity, can be prepared using routine recombinant DNA manipulations as well as random or site-directed mutagenesis techniques. Furthermore, hER β -derived variants or fragments that exhibit transcriptional activation activity can be identified using routine 5 experimentation by employing the methods described herein, e.g., by co-expression with an appropriate reporter gene followed by measurement of reporter gene transcription in the presence and absence of an estrogen.

In another embodiment, the present invention encompasses isolated, purified, nucleic acids comprising nucleotides 94-229 of the sequence 10 depicted in Figure 3, SEQ ID NO:1, which encode amino acids 1-45 of hER β , and sequence-conservative variants thereof.

The nucleic acids of the present invention include purine- and pyrimidine-containing polymers of any length, either polyribonucleotides or polydeoxyribonucleotides or mixed polyribo-polydeoxyribo nucleotides. This 15 includes single- and double-stranded molecules, i.e., DNA-DNA, DNA-RNA and RNA-RNA hybrids, as well as "protein nucleic acids" (PNA) formed by conjugating bases to an amino acid backbone. This also includes nucleic acids containing modified bases.

The nucleic acids may be isolated directly from cells. Alternatively, 20 PCR can be used to produce the nucleic acids of the invention, using either chemically synthesized strands or genomic material as templates. Primers used for PCR can be synthesized using the sequence information provided herein and can further be designed to introduce appropriate new restriction sites, if desirable, to facilitate incorporation into a given vector for 25 recombinant expression.

The nucleic acids of the present invention may be flanked by natural regulatory sequences, or may be associated with heterologous sequences, including promoters, enhancers, response elements, signal sequences, polyadenylation sequences, introns, 5'- and 3'- noncoding regions, and the 30 like. The nucleic acids may also be modified by many means known in the art. Non-limiting examples of such modifications include methylation,

"caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoroamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.). Nucleic acids may contain one or more additional covalently linked moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), intercalators (e.g., acridine, psoralen, etc.), chelators (e.g., metals, radioactive metals, iron, oxidative metals, etc.), and alkylators. PNAs are also included. The nucleic acid may be derivatized by formation of a methyl or ethyl phosphotriester or an alkyl phosphoramidate linkage. Furthermore, the nucleic acid sequences of the present invention may also be modified with a label capable of providing a detectable signal, either directly or indirectly. Exemplary labels include radioisotopes, fluorescent molecules, biotin, and the like.

The invention also provides nucleic acid vectors comprising hER β -encoding sequences or derivatives or fragments thereof. A large number of vectors, including plasmid and fungal vectors, have been described for replication and/or expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple cloning or protein expression. The encoded hER β -derived polypeptides may be expressed by using many known vectors, such as pUC plasmids, pET plasmids (Novagen, Inc., Madison, WI), or pRSET or pREP (Invitrogen, San Diego, CA), and many appropriate host cells, using methods disclosed or cited herein or otherwise known to those skilled in the relevant art. The particular choice of vector/host is not critical to the practice of the invention.

Recombinant cloning vectors will often include one or more replication systems for cloning or expression, one or more markers for selection in the host, e.g. antibiotic resistance, and one or more expression cassettes. The inserted hER β -encoding sequences may be synthesized by

standard methods, isolated from natural sources, or prepared as hybrids, etc. Ligation of the hER β -encoding sequences to transcriptional regulatory elements and/or to other amino acid coding sequences may be achieved by known methods. Suitable host cells may be

5 transformed/transfected/infected as appropriate by any suitable method including electroporation, CaCl₂ mediated DNA uptake, viral vector-mediated DNA delivery, fungal infection, microinjection, microprojectile, or other established methods.

Appropriate host cells included bacteria, archebacteria, fungi,
10 especially yeast, and plant and animal cells, especially mammalian cells. Of particular interest are E. coli, B. Subtilis, Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Schizosaccharomyces pombe, SF9 cells, C129 cells, 293 cells, Neurospora, and HepG2 cells, CHO cells, COS cells, HeLa cells, and immortalized mammalian myeloid and lymphoid cell lines.
15 Preferred replication systems include M13, ColE1, SV40, baculovirus, lambda, adenovirus, and the like. A large number of transcription initiation and termination regulatory regions have been isolated and shown to be effective in the transcription and translation of heterologous proteins in the various hosts. Examples of these regions, methods of isolation, manner of
20 manipulation, etc. are known in the art. Under appropriate expression conditions, host cells can be used as a source of recombinantly produced hER β -derived peptides and polypeptides.

Advantageously, vectors may also include a transcription regulatory element (i.e., a promoter) operably linked to the hER β portion. The
25 promoter may optionally contain operator portions and/or ribosome binding sites. Non-limiting examples of bacterial promoters compatible with E. coli include: β -lactamase (penicillinase) promoter; lactose promoter; tryptophan (trp) promoter; arabinose BAD operon promoter; lambda-derived PI promoter and N gene ribosome binding site; and the hybrid tac promoter derived from
30 sequences of the trp and lac UV5 promoters. Non-limiting examples of yeast promoters include triose phosphate isomerase promoter, 3-

phosphoglycerate kinase promoter, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter, galactokinase (GAL1) promoter, galactoepimerase promoter, and alcohol dehydrogenase (ADH) promoter.

5 Suitable promoters for mammalian cells include without limitation viral promoters such as that from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Mammalian cells may also require terminator sequences and poly A addition sequences and enhancer sequences which increase expression may also be included; sequences which cause amplification of the gene may also be desirable.

10 Furthermore, sequences that facilitate secretion of the recombinant product from cells, including, but not limited to, bacteria, yeast, and animal cells, such as secretory signal sequences and/or prohormone pro region sequences, may also be included. These sequences are well described in the art.

15 Nucleic acids encoding hER β -derived polypeptides may also be introduced into cells by recombination events. For example, such a sequence can be introduced into a cell, and thereby effect homologous recombination at the site of an endogenous gene or a sequence with substantial identity to the gene. Other recombination-based methods such

20 as nonhomologous recombinations or deletion of endogenous genes by homologous recombination may also be used.

The nucleic acids of the present invention find use as templates for the recombinant production of hER β -derived peptides or polypeptides.

25 **hER β -derived polypeptides**

The present invention encompasses purified hER β -derived polypeptides comprising amino acids 1-45 of hER β and further comprising all or part of the amino acid sequence depicted in Figure 4, SEQ ID NO:2, and function-conservative variants thereof, i.e., variants that exhibit

30 estrogen-induced transcriptional activation activity. Also encompassed by

the invention are peptides comprising amino acids 1-45 of SEQ ID NO:2 and function-conservative variants thereof.

Nucleic acids comprising hER β -coding sequences can be used to direct the expression of hER β -derived polypeptides in intact cells or in cell-free translation systems. The known genetic code, tailored if desired for more efficient expression in a given host organism, can be used to synthesize oligonucleotides encoding the desired amino acid sequences. The phosphoramidite solid support method of Matteucci et al., 1981, J. Am. Chem. Soc. 103:3185, the method of Yoo et al., 1989, J. Biol. Chem. 10 764:17078, or other well known methods can be used for such synthesis. The resulting oligonucleotides can be inserted into an appropriate vector and expressed in a compatible host organism.

The polypeptides of the present invention, including function-conservative variants of the disclosed hER β sequences, may be isolated from wild-type or mutant human cells, or from heterologous organisms or cells (including, but not limited to, bacteria, fungi, insect, plant, and mammalian cells) into which an hER β -derived protein-coding sequence has been introduced and expressed. Furthermore, the polypeptides may be part of recombinant fusion proteins.

20 Polypeptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The polypeptides are preferably prepared by solid phase peptide synthesis as described by Merrifield, 1963, J. Am.

25 Chem. Soc. 85:2149.

"Isolation" or "purification" of an hER β -derived polypeptide refers to the isolation of the polypeptide in a form that allows its transcriptional activation activity to be measured without interference by other components of the cell in which the polypeptide is expressed. Methods for 30 polypeptide purification are well-known in the art, including, without limitation, preparative disc-gel electrophoresis, isoelectric focusing, HPLC,

reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, and countercurrent distribution. For some purposes, it is preferable to produce the polypeptide in a recombinant system in which the hER β -derived protein contains an additional sequence tag that facilitates purification, such as, but not limited to, a polyhistidine sequence. The polypeptide can then be purified from a crude lysate of the host cell by chromatography on an appropriate solid-phase matrix. Alternatively, antibodies produced against an hER β -derived protein or against peptides derived therefrom can be used as purification reagents. Other purification methods are possible.

The present invention also encompasses derivatives and homologues of hER β -encoded polypeptides. For some purposes, nucleic acid sequences encoding the peptides may be altered by substitutions, additions, or deletions that provide for functionally equivalent molecules, i.e., function-conservative variants. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of similar properties, such as, for example, positively charged amino acids (arginine, lysine, and histidine); negatively charged amino acids (aspartate and glutamate); polar neutral amino acids; and non-polar amino acids.

The isolated polypeptides may be modified by, for example, phosphorylation, sulfation, acylation, or other protein modifications. They may also be modified with a label capable of providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotopes and fluorescent compounds.

25

hER β -specific Antibodies

The present invention encompasses antibodies that specifically recognize hER β -derived peptides and polypeptides, including without limitation antibodies that recognize hER β but not, e.g., hER α , and those that recognize hER β L but not hER β T. Such hER β -specific antibodies can be used conventionally, e.g., as diagnostic reagents or as reagents for purification

of hER β -derived polypeptides. Other uses include immunocytochemical localization of hER β ; gel shift assays; and "pull-down" experiments to identify protein co-activators associated with hER β .

hER β -specific antibodies according to the present invention include 5 polyclonal and monoclonal antibodies. The antibodies may be elicited in an animal host by immunization with hER β immunogenic components or may be formed by in vitro immunization (sensitization) of immune cells. The immunogenic components used to elicit the production of antibodies may be isolated from any cell source or may be chemically synthesized. The 10 antibodies may also be produced in recombinant systems programmed with appropriate antibody-encoding DNA. Alternatively, the antibodies may be constructed by biochemical reconstitution of purified heavy and light chains. The antibodies include hybrid antibodies (i.e., containing two sets of heavy chain/light chain combinations, each of which recognizes a 15 different antigen), chimeric antibodies (i.e., in which either the heavy chains, light chains, or both, are fusion proteins), and univalent antibodies (i.e., comprised of a heavy chain/light chain complex bound to the constant region of a second heavy chain). Also included are Fab fragments, including Fab' and F(ab)2 fragments of antibodies. Methods for the 20 production of all of the above types of antibodies and derivatives are well-known in the art. For example, techniques for producing and processing polyclonal antisera are disclosed in Mayer and Walker, 1987, Immunochemical Methods in Cell and Molecular Biology, (Academic Press, London). The general methodology for making monoclonal antibodies by 25 hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., Schreier et al., 1980, Hybridoma Techniques. Panels of monoclonal antibodies produced against hER β epitopes can be 30 screened for various properties; i.e., for isotype, epitope affinity, etc.

Antibodies against hER β -derived immunogenic components can be used, unlabeled or labeled by standard methods, as the basis for immunoassays. The particular label used will depend upon the type of immunoassay used. Examples of labels that can be used include but are 5 not limited to radiolabels such as ^{32}P , ^{125}I , 3H and ^{14}C ; fluorescent labels such as fluorescein and its derivatives, rhodamine and its derivatives, dansyl and umbelliflone; chemiluminescers such as luciferase and 2,3-dihydropthal-azinediones; and enzymes such as horseradish peroxidase, alkaline phosphatase, lysozyme and glucose-6-phosphate dehydrogenase.

10

Applications

The methods and compositions of the present invention can be used to identify compounds that interact with hER β , either to activate or to inhibit its transcriptional activation function. Such compounds include, 15 without limitation, co-activator proteins, as well as estrogens and other steroids, steroid-like molecules, or non-steroid-like molecules that act as agonists or antagonists. Screening methods can also be used to identify tissue-specific estrogens.

Identification of hER β -interactive compounds can be achieved by 20 cell-free or cell-based assays. In one set of embodiments, purified hER β is contacted with a labelled ligand, such as, e.g., $^{17}\beta$ estradiol, in the presence of test compounds to form test reactions, and in the absence of test compounds to form control reactions. The labelled moiety may comprise a radiolabel (such as, e.g., 3H or ^{125}I) or a fluorescent molecule. 25 Incubation is allowed to proceed for a sufficient time and under appropriate conditions to achieve specific binding, after which binding of labelled estradiol to hER β is measured (by monitoring, e.g., radioactivity, fluorescence, or fluorescence polarization). In one embodiment, hER β produced in *E. coli* (as described in Example 1 below) is adsorbed to the 30 wells of a microtiter dish and incubated with [3H]- $^{17}\beta$ estradiol in the absence or presence of test compounds (see, e.g., Example 2 below).

Alternatively, soluble receptor is incubated with the labelled ligand in the absence or presence of test compounds, and bound ligand is separated from free ligand, either by filtration on glass fiber filters or by using dextran-coated charcoal. See, e.g., Hulme, ed., Receptor-ligand Interactions: A

5 Practical Approach, IRL Press, NY, 1992).

Whole cell binding assays may also be used in which bound ligand is separated from free ligand by rinsing. Cells used in these assays may either contain endogenous receptor, or may overexpress the receptor subsequent to stable or transient transfection or infection of an hER β gene or cDNA.

10 Non-limiting examples of suitable cells include COS cells, Hela cells, CHO cells, human umbilical vein endothelial cells (HUVEC), and yeast. Once a compound has been identified as an hER β -interactive compound by its binding activity, further *in vivo* and *in vitro* tests may be performed to determine the nature and extent of activity, i.e., as an agonist or antagonist
15 (see below).

hER β -interactive compounds may also be identified using cell-based assays that measure transcriptional activation or suppression of endogenous or transfected estrogen-responsive genes. For example, agonists (such as, e.g., 17 β -estradiol) block interleukin-1b induction of
20 endogenous E-selectin in primary human umbilical vein endothelial cells (HUVEC) that express hER β . Antagonists (such as, e.g., ICI-182780) block the agonist activity of 17 β -estradiol. Non-limiting examples of other suitable endogenous estrogen-responsive promoter elements include those that regulate endothelin-1 (ET-1); HDL receptor (scavenger receptor type II);
25 and enzymes involved in coagulation and fibrinolysis (such as, e.g., plasminogen activator inhibitor-1 and complement C3). Any promoter element that responds to estrogen may be used as an appropriate target, including, e.g., the NF κ B binding site or the apolipoprotein A1 gene enhancer sequence.
30 In one set of embodiments, appropriate host cells are transfected with an expression vector encoding hER β and the transfectants are

incubated with or without estradiol in the presence or absence of test compounds. hER β activity is assessed by measuring transcriptional activation of the target sequence. This may be achieved by detection of mRNA (using, e.g., Northern blot analysis) and/or by detection of the protein (using, e.g., immunoassays or functional assays). If activation of the target sequence initiates a biochemical cascade, downstream biological events may also be measured to quantify hER β activity. hER β -interactive compounds are identified as those that positively or negatively influence target sequence activation.

10 In another set of embodiments, appropriate host cells (preferably, bacterial or yeast cells) are co-transfected with an expression vector encoding hER β and a reporter plasmid containing a reporter gene downstream of one or more estrogen response elements (EREs). Transfected cells are incubated with or without estradiol in the presence of

15 absence of test compounds, after which hER β activity is determined by measuring expression of the reporter gene. In a preferred embodiment, hER β activity is monitored visually. Non-limiting examples of suitable reporter genes include luciferase, chloramphenicol acetyl transferase (CAT), and green fluorescence protein.

20 Preferably, the methods of the present invention are adapted to a high-throughput screen, allowing a multiplicity of compounds to be tested in a single assay. Candidate estrogens and estrogen-like compounds include without limitation diethylstilbestrol, genistein, and estrone. Other hER β -interactive compounds may be found in, for example, natural product

25 libraries, fermentation libraries (encompassing plants and microorganisms), combinatorial libraries, compound files, and synthetic compound libraries. For example, synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates (Merrimack, NH), and Microsource (New Milford,

30 CT). A rare chemical library is available from Aldrich Chemical Company, Inc. (Milwaukee, WI). Alternatively, libraries of natural compounds in the

form of bacterial, fungal, plant and animal extracts are available from, for example, Pan Laboratories (Bothell, WA) or MycoSearch (NC), or are readily producible. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, 5 and biochemical means (Blondelle et al., *TibTech* **14**:60, 1996). hER β binding assays according to the present invention are advantageous in accommodating many different types of solvents and thus allowing the testing of compounds from many sources.

Compounds identified as hER β agonists or antagonists using the 10 methods of the present invention may be modified to enhance potency, efficacy, uptake, stability, and suitability for use in therapeutic applications, etc. These modifications are achieved and tested using methods well-known in the art.

15 **Description of the Preferred Embodiments**

The following examples are intended to illustrate the present invention without limitation.

Example 1: High-level Expression of Human hER β in *E. coli*

20 Human hER β according to the present invention is overexpressed in *E. coli* strain BL21(DE3) using, for example, the pET15B vector. A 10-ml overnight culture is used to inoculate 1 liter LB medium containing 100 mg/ml ampicillin. Cultures are grown at 37°C and then induced by the addition of 1 mM IPTG. After an additional incubation for 2h at 25°C, cells 25 are harvested by centrifugation at 10,000 X G for 30 minutes and resuspended in 100 ml of a buffer containing 50 mM Tris-HCl, pH 7.4 -150 mM NaCl. Cells are lysed using a French press, and insoluble material is pelleted by centrifugation. The supernatant solution is recovered and stored at -70°C.

Example 2: Estrogen Receptor- β Ligand Binding Assay

For determining the ability of a particular compound to bind hER β , 100 ml of the receptor preparation described in Example 1 above, diluted in assay buffer (Dulbecco's phosphate buffered saline (Gibco #14200-075) 5 supplemented with 1 mM EDTA), is added to each well of a high-binding masked microtiter plate (Wallac #1450-511, Gaithersburg MD). 10 ml of test compound (or vehicle) and 10 ml of [³H]-17 β -estradiol are added to each well, and the plate is incubated at room temperature for 4-6 hours. Unbound material is aspirated, and the plate is washed three times with 10 300 ml of assay buffer. Then, 150 ml of scintillation cocktail (Optiphase Supermix, Wallac #1200-439) is added per well, and the plate is sealed and agitated for at least 5 min. Bound radioactivity is measured by scintillation counting.

Test compounds are initially tested at a concentration of 1.5 mg/ml 15 (approximately 5 mM for a compound having a molecular mass of 300). Positive compounds are then re-tested at a number of different concentrations to determine the IC₅₀.

Data are expressed as percent inhibition of specific binding. Exploratory data analysis (EDA) is performed on raw data to check for non- 20 normality and non-homogeneity of variance. The maximum likelihood Box-Cox transformation, which maximizes the normality, homogeneity of variance, and goodness of fit of the data, is then obtained. Based on the result, the appropriate transformation of the data (no transformation, square root transformation, or logarithmic transformation) is used for model fitting. 25 The Huber M-estimator is used to down weight any outlying transformed observations for analysis of variance and dose-response curve fitting.

For ANOVA, multiple comparisons LSD p-values are computed. Re-transformed summary statistics (mean, s.d., s.e.m.) are obtained for each treatment group.

30 For dose-response curve fitting, a four parameter logistic model on the transformed, weighted data are fit. The four parameters are min, max,

slope, and ED50, where ED50 is defined as the dose which corresponds to midway between the estimated max and min. All of the parameters and confidence intervals are re-transformed back to the original units of the data. A further transformation into percent inhibition (using estimated min
5 and max) is performed.

Using this assay, the following values were obtained for reference compounds:

		<u>IC50</u>	<u>95% confidence limits</u>
10	17 β -estradiol	6.7 nM	6 - 7.5 nM
	diethylstilbestrol	21 nM	14 - 31 nM
	genistein	1.6 nM	1.4 - 1.8 nM

All patents, applications, articles, publications, and test methods mentioned above are hereby incorporated by reference.

15

Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed description. Such obvious variations are within the full intended scope of the appended claims.

Claims:

1. An isolated nucleic acid comprising the sequence depicted in Figure 1, SEQ ID NO:1.
2. A nucleic acid as defined in claim 1, wherein said nucleic acid is DNA.
3. A nucleic acid as defined in claim 1, wherein said nucleic acid is RNA.
4. A recombinant DNA vector comprising a sequence as defined in claim 1.
5. A recombinant DNA vector comprising a sequence as defined in claim 1 operably linked to a transcription regulatory element.
6. A cell comprising a DNA vector as defined in claim 5, wherein said cell is selected from the group consisting of bacterial, fungal, plant, insect, and mammalian cells.
7. A method for producing a polypeptide, said method comprising incubating a cell as defined in claim 6 under conditions that permit expression of one or more polypeptides encoded by said nucleic acid.
8. A method as defined in claim 7, further comprising:
 - (a) harvesting said incubated cells to produce a cell fraction and a medium fraction; and
 - (b) recovering said one or more polypeptides from said cell fraction, said medium fraction, or both.
9. A purified isolated nucleic acid encoding the amino acid sequence depicted in Figure 1 SEQ ID NO:2.
10. A nucleic acid as defined in claim 9, wherein said nucleic acid is DNA.
11. A nucleic acid as defined in claim 9, wherein said nucleic acid is RNA.
12. A recombinant DNA vector comprising a sequence as defined in claim 9.

28

13. A recombinant DNA vector comprising a sequence as defined in claim 9 operably linked to a transcription regulatory element.

14. A cell comprising a DNA vector as defined in claim 13, wherein said cell is selected from the group consisting of bacterial, fungal, plant, insect, and mammalian cells.

15. A method for producing a polypeptide, said method comprising incubating a cell as defined in claim 14 under conditions that permit expression of one or more polypeptides encoded by said nucleic acid.

16. A method as defined in claim 15, further comprising:

(a) harvesting said incubated cells to produce a cell fraction and a medium fraction; and

(b) recovering said one or more polypeptides from said cell fraction, said medium fraction, or both.

17. A purified polypeptide comprising a sequence selected from the group consisting of the sequence depicted in Figure 1 SEQ ID NO:2 and function-conservative variants thereof.

18. A purified polypeptide comprising amino acids 1-45 of the sequence depicted in Figure 1 SEQ ID NO:2.

19. A method for identifying hER β -interactive compounds, said method comprising:

(a) contacting purified hER β with a labelled ligand in the presence of test compounds, to form test reactions, and in the absence of test compounds, to form control reactions;

(b) incubating said test and control reactions under appropriate conditions to achieve equilibrium binding of said labelled ligand to hER β ;

(c) determining the level of binding of said labelled ligand to hER β in said test and control cultures; and

(d) identifying as a hER β -interactive compound any compound that reduces the binding of said labelled ligand to hER β .

29

20. A method as defined in claim 19, wherein said ligand is 17- β estradiol.
21. A method as defined in claim 19, wherein said hER β -interactive compound is an agonist.
22. A method as defined in claim 19, wherein said hER β -interactive compound is an antagonist.
23. An antibody that specifically recognizes hER β .

1/9

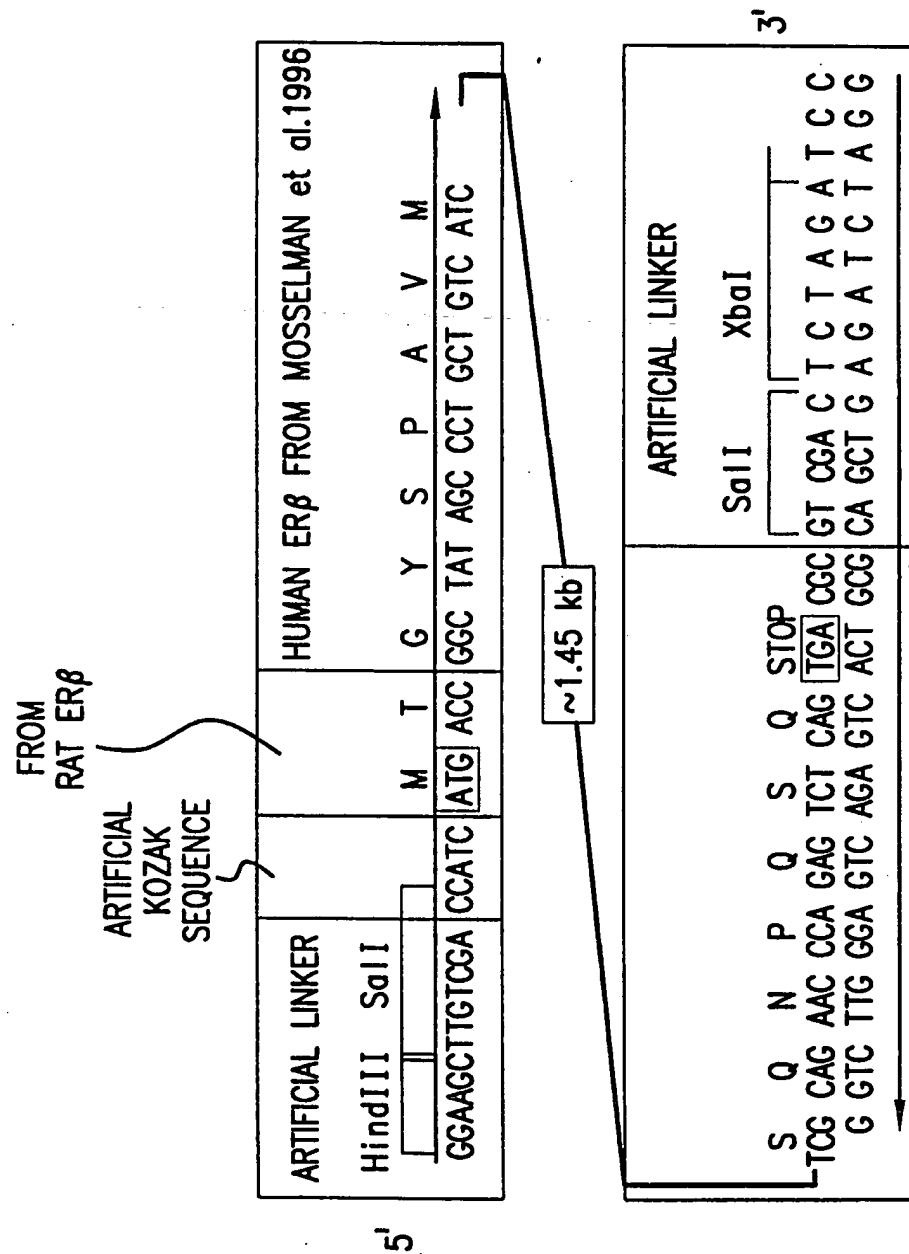


FIG. 1

2/9

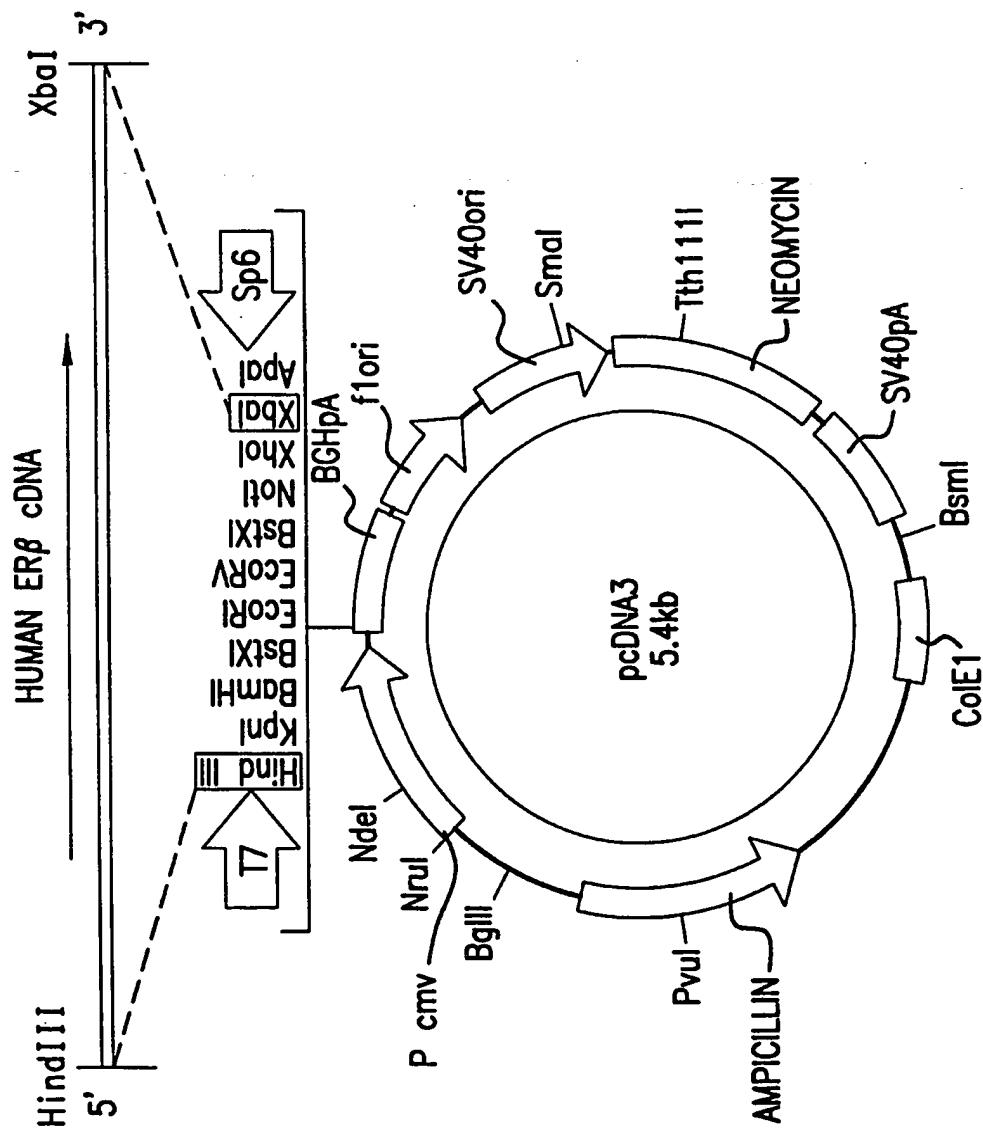


FIG. 2

3/9

CAGCCATTATACTTGCACGAACTTGAGAACATTATAATGACCTTGCGCTCTCT 60
TGCAAGGTGTTTCTCAGCTGCTATCTCAAGACATGGATATAAAAAACTCACCATCTAGC 120
CTTAATTCTCCTCCTACAACTGCAGTCATCCATCTTACCCCTGGAGCACGGCTCC 180
ATATACATACCTCCTCCTATGAGACAGCCACCATGAATATCCAGCCATGACATTCTAT 240
AGCCCTGCTGTGATGAATTACAGCATTCCCAGCAATGTCACTAACTTGGAGGTGGCCT 300
GGTCGGCAGACCACAAGCCAAATGTGTTGGCCAACACCTGGGACCTTCTCCTTA 360
GTGGTCCATGCCAGTTATCACATCTGATGCGAACCTCAAAAGAGTCCCTGGTGTGAA 420
GCAAGATCGCTAGAACACACCTTACCTGTAACAGAGAGACACTGAAAAGGAAGGTTAGT 480
GGGAACCGTTGCGCCAGCCCTGTTACTGGTCCAGGTTCAAAGAGGGATGCTCACTCTGC 540
GCTGTCTGCAGCGATTACGCATGGATATCACTATGGAGTCTGGCGTGTGAAGGATGT 600
AAGGCCTTTTAAAAGAACATTCAAGGACATAATGATTATATTGTCCAGCTACAAAT 660
CA GTACAATCGATAAAACCGCGCAAGAGCTGCCAGGCCTGCCACTTCGGAAGTGT 720
TACGAAGTGGGAATGGTGAAGTGTGGCTCCGGAGAGAGAGATGTGGGTACCGCCTTG 780
CGGAGACAGAGAAGTGCCGACGAGCAGCTGCACTGTGCCGGCAAGGCCAAGAGAAGTGGC 840
GGCCACCGCGCCCCGAGTGCGGGAGCTGCTGCTGGACGCCCTGAGCCCCGAGCAGCTAGTG 900
CTCACCCCTCTGGAGGCTGAGCCGCCCCATGTGCTGATCAGCCGCCCCAGTGCGCCCTTC 960
ACCGAGGCCTCCATGATGATGTCCCCTGACCAAGTTGGCGACAAGGAGTTGGTACACATG 1020
ATCAGCTGGCCAAGAACGATTCCGGCTTGTGGAGCTCAGCCTGTCGACCAAGTGC 1080
CTCTGGAGAGCTGTTGGATGGAGGTGTTAATGATGGGCTGATGTGGCGCTCAATTGAC 1140
CACCCCGCAAGCTCATCTTGCTCCAGATCTGTTCTGGACAGGGATGAGGGAAATGC 1200
GTAGAAGGAATTCTGAAATCTTGACATGCTCTGGCAACTACTTCAAGGTTCGAGAG 1260
TTAAAACCAACACAAAGAATATCTCTGTCAGGCCATGATCCTGCTCAATTCCAGT 1320
ATGTACCCCTCTGGTACAGCGACCCAGGATGCTGACAGCAGCCGGAAAGCTGGCTCACTTG 1380
CTGAACGCCGTGACCGATGCTTGGTTGGTGATTGCCAAGAGCGGCATCTCCTCCCAG 1440
CAGCAATCCATGCCCTGGCTAACCTCCTGATGCTCCTGCCCACGTCAAGGCATGCGAGT 1500
AACAAAGGGCATGGAACATCTGCTCAACATGAAGTGCAAAATGTGGTCCAGTGTATGAC 1560
CTGCTGCTGGAGATGCTGAATGCCACGTGCTCGCGGGTGCAAGTCCTCCATCACGGGG 1620
TCCGAGTGCAGCCGGCAGAGGACAGTAAAGCAAAGAGGGCTCCAGAACCCACAGTCT 1680
CAGTGA 1686

FIG.3
SUBSTITUTE SHEET (RULE 26)

4/9

<u>MDIKNSPSSL</u>	NSPSSYNC <u>SO</u>	SILPLEHGSI	YIPSSYVDSH	HEYPAMTFYS	50
PAVMNYSIPS	NVTNLEGGPG	RQTTSPNVLW	PTPGHLSPLV	VHRQLSHLYA	100
EPQKSPWCEA	RSLEHTLPVN	RETLKRKVSG	NRCASPVTGP	GSKRDAHFCA	150
VCSDYASGYH	YGVWSCEGCK	AFFKRSIQGH	NDYICPATNQ	CTIDKNRRKS	200
CQACRLRKCY	EVGMVKCGSR	RERCGYRLVR	RQRSADEQLH	CAGKAKRSGG	250
HAPRVRELLL	DALSPEQLVL	TLLEAEPPHV	LISRPSAPFT	EASMMMSLTK	300
LADKELVHMI	SWAKKIPGFV	ELSLFDQVRL	LESCWMEVLM	MGLMWRSIDH	350
PGKLIFAPDL	VLDRDEGKCV	EGILEIFDML	LATTSRFREL	KLQHKEYLCV	400
KAMILNNSSM	YPLVTATQDA	DSSRKLAHLL	NAVTDALVWV	IAKSGISSLQQ	450
QSMRLANLLM	LLSHVRHASN	KGMHLLNMK	CKNVVPVYDL	LLEMLNAHVL	500
RGCKSSITGS	ECSPAEDSKS	KEGSQNPQSQ			531

FIG.4

5/9

1 2 3 M

- 221

- 133

- 93

- 67

- 56

- 42

- 28

FIG.5

6/9

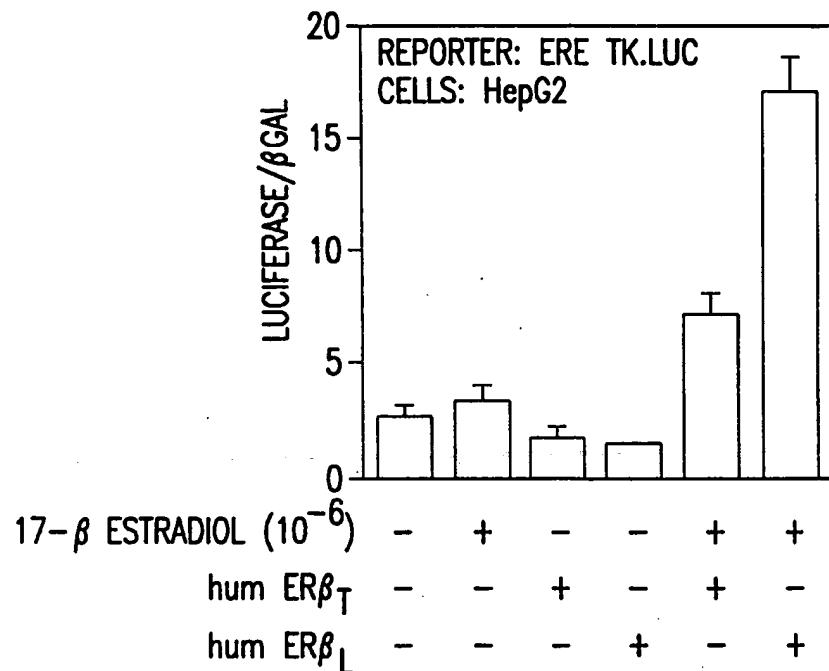


FIG.6A

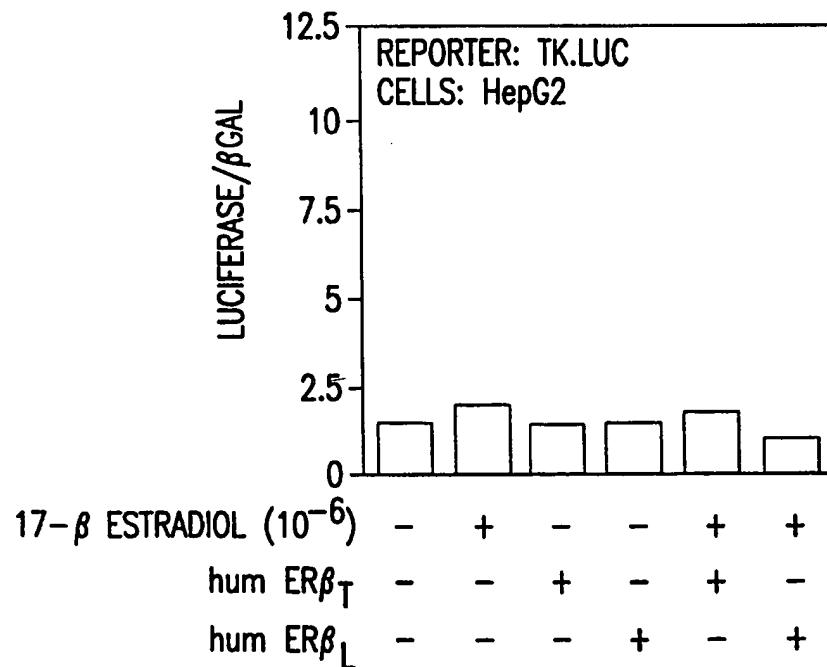


FIG.6B

SUBSTITUTE SHEET (RULE 26)

7/9

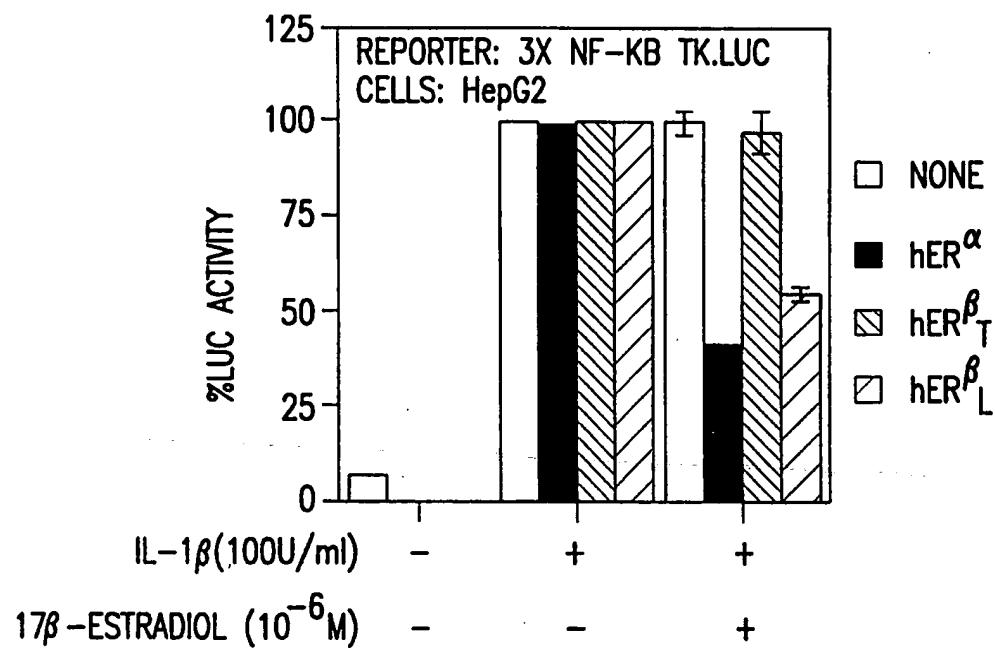
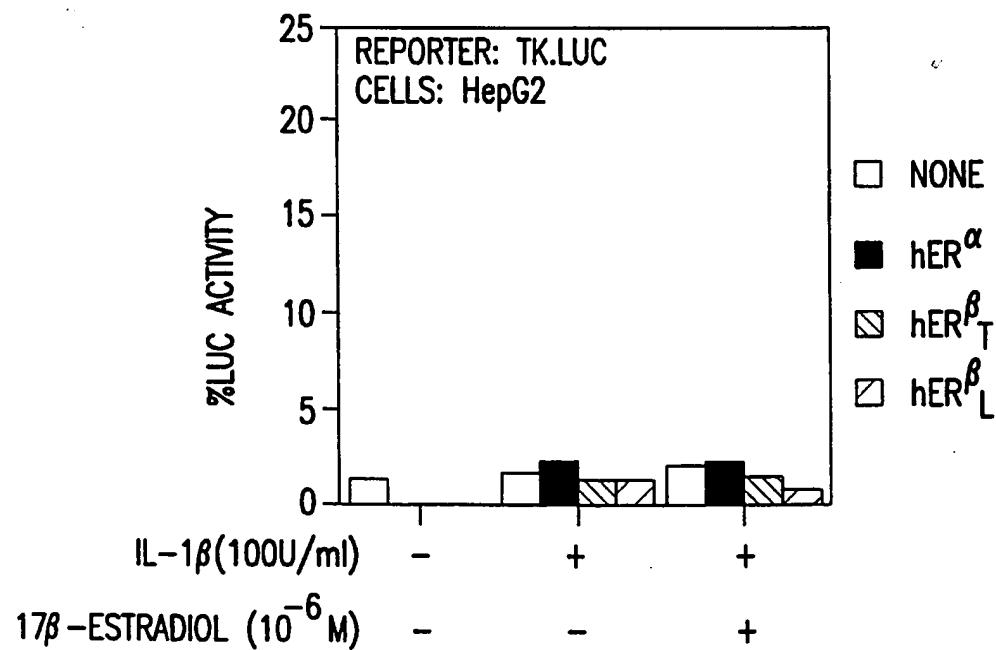


FIG.7A

FIG.7B
SUBSTITUTE SHEET (RULE 26)

8/9

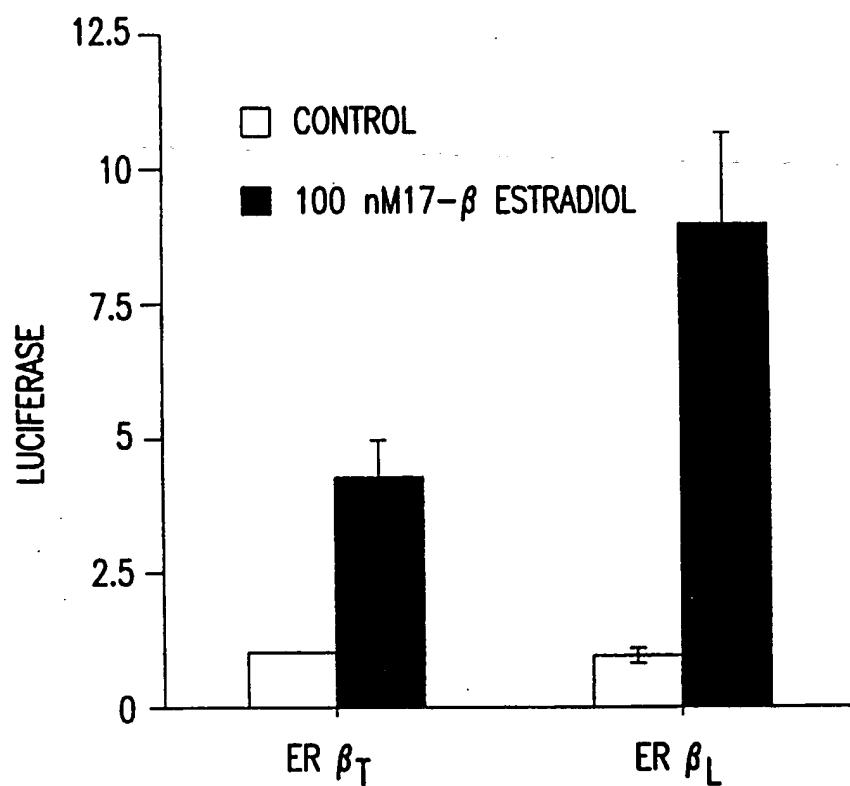


FIG.8

9/9

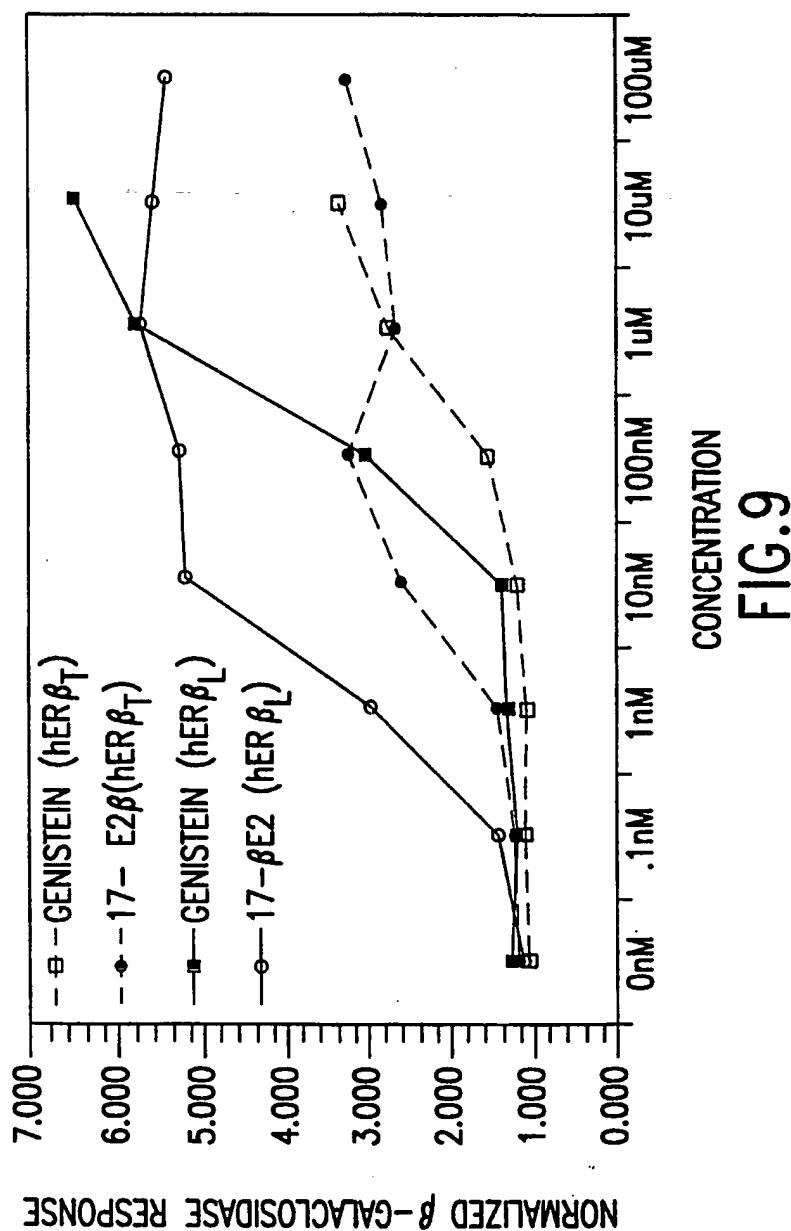


FIG. 9

1/7

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: American Home Products Corporation
- (B) STREET: Five Giralda Farms
- (C) CITY: Madison
- (D) STATE: New Jersey
- (E) COUNTRY: U.S.A.
- (F) POSTAL CODE (ZIP): 07940
- (G) TELEPHONE: (973) 683-2169
- (H) TELEFAX: (973) 683-4117

(ii) TITLE OF INVENTION: Novel Human Estrogen Receptor-B

(iii) NUMBER OF SEQUENCES: 13

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1686 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CAGCCATTAT ACTTGCCCCAC GAATCTTGAA GAACATTATA ATGACCTTTG TGCCTCTTCT	60
TGCAAGGTGT TTTCTCAGCT GCTATCTCAA GACATGGATA TAAAAAAACTC ACCATCTAGC	120
CTTAATTCTC CTTCCCTCCA CAACTGCAGT CAATCCATCT TACCCCTGGA GCACGGCTCC	180
ATATACATAC CTTCCCTCCA TGTAGACAGC CACCATGAAT ATCCAGCCAT GACATTCTAT	240
AGCCCTGCTG TGATGAATTA CAGCATTCCC AGCAATGTCA CTAACATTGGA AGGTGGGCCT	300
GGTCGGCAGA CCACAAGCCC AAATGTGTTG TGGCCAACAC CTGGGCACCT TTCTCCTTTA	360
GTGGTCCATC GCCAGTTATC ACATCTGTAT GCGGAACCTC AAAAGAGTCC CTGGTGTGAA	420
GCAAGATCGC TAGAACACAC CTTACCTGTA AACAGAGAGA CACTGAAAAG GAAGGTTAGT	480
GGGAACCGTT GCGCCAGCCC TGTTACTGGT CCAGGTTCAA AGAGGGATGC TCACCTCTGC	540
GCTGTCTGCA GCGATTACGC ATCGGGATAT CACTATGGAG TCTGGTCGTG TGAAGGATGT	600
AAGGCCTTTT TTAAAAGAAG CATTCAAGGA CATAATGATT ATATTTGTCC AGCTACAAAT	660
CAGTGTACAA TCGATAAAAAA CGGGCGCAAG AGCTGCCAGG CCTGCCGACT TCGGAAGTGT	720

2/7

TACGAAGTGG	GAATGGTGAA	GTGTGGCTCC	CGGAGAGAGA	GATGTGGTA	CCGCCTTGTG	780
CGGAGACAGA	GAAGTGCCGA	CGAGCAGCTG	CACTGTGCCG	GCAAGGCCAA	GAGAAGTGGC	840
GGCCACGCGC	CCCGAGTGC	GGAGCTGCTG	CTGGACGCC	TGAGCCCCGA	GCAGCTAGTG	900
CTCACCCCTCC	TGGAGGCTGA	GCCGCCCAT	GTGCTGATCA	GCCGCCAG	TGCCCCCTTC	960
ACCGAGGCCT	CCATGATGAT	GTCCCCTGACC	AAGTTGCCG	ACAAGGAGTT	GGTACACATG	1020
ATCAGCTGGG	CCAAGAAGAT	TCCCAGCTTT	GTGGAGCTCA	GCCTGTTGCA	CCAAGTGC	1080
CTCTTGGAGA	GCTGTTGGAT	GGAGGTGTTA	ATGATGGGGC	TGATGTGGCG	CTCAATTGAC	1140
CACCCCGGCA	AGCTCATCTT	TGCTCCAGAT	CTTGGTCTGG	ACAGGGATGA	GGGGAAATGC	1200
GTAGAAGGAA	TTCTGAAAT	CTTGACATG	CTCCTGGCAA	CTACTTCAAG	GTTTCGAGAG	1260
TTAAAACCTCC	AACACAAAGA	ATATCTCTGT	GTCAAGGCCA	TGATCCTGCT	CAATTCCAGT	1320
ATGTACCCCTC	TGGTCACAGC	GACCCAGGAT	GCTGACAGCA	GCCGGAAGCT	GGCTCACTTG	1380
CTGAACGCCG	TGACCGATGC	TTTGGTTGG	GTGATTGCCA	AGAGCGGCAT	CTCCTCCCAG	1440
CAGCAATCCA	TGCGCCTGGC	TAACCTCCTG	ATGCTCCTGT	CCCACGTCAG	GCATGCGAGT	1500
AACAAGGGCA	TGGAACATCT	GCTAACATG	AAGTGCAAA	ATGTGGTCCC	AGTGTATGAC	1560
CTGCTGCTGG	AGATGCTGAA	TGCCCACGTG	CTTCGCGGGT	GCAAGTCCTC	CATCACGGGG	1620
TCCGAGTGCA	GCCCAGCAGA	GGACAGTAAA	AGCAAAGAGG	GCTCCCAGAA	CCACAGTCT	1680
CAGTGA						1686

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 530 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met	Asp	Ile	Lys	Asn	Ser	Pro	Ser	Ser	L	Asn	Ser	Pro	Ser	Ser	Tyr
1									5						15
Asn	Cys	Ser	Gln	Ser	Ile	Leu	Pro	Leu	Glu	His	Gly	Ser	Ile	Tyr	Ile
									20			25		30	
Pro	Ser	Ser	Tyr	Val	Asp	Ser	His	His	Glu	Tyr	Pro	Ala	Met	Thr	Phe
									35			40		45	
Tyr	Ser	Pro	Ala	Val	Met	Asn	Tyr	Ser	Ile	Pro	Ser	Asn	Val	Thr	Asn
									50			55		60	

3/7

Leu Glu Gly Gly Pro Gly Arg Gln Thr Thr Ser Pro Asn Val Leu Trp
 65 70 75 80
 Pro Thr Pro Gly His Leu Ser Pro Leu Val Val His Arg Gln Leu Ser
 85 90 95
 His Leu Tyr Ala Glu Pro Gln Lys Ser Pro Trp Cys Glu Ala Arg Ser
 100 105 110
 Leu Glu His Thr Leu Pro Val Asn Arg Glu Thr Leu Lys Arg Lys Val
 115 120 125
 Ser Gly Asn Arg Cys Ala Ser Pro Val Thr Gly Pro Gly Ser Lys Arg
 130 135 140
 Asp Ala His Phe Cys Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His
 145 150 155 160
 Tyr Gly Val Trp Ser Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser
 165 170 175
 Ile Gln Gly His Asn Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr
 180 185 190
 Ile Asp Lys Asn Arg Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys
 195 200 205
 Cys Tyr Glu Val Gly Met Val Lys Cys Gly Ser Arg Arg Glu Arg Cys
 210 215 220
 Gly Tyr Arg Leu Val Arg Arg Gln Arg Ser Ala Asp Glu Gln Leu His
 225 230 235 240
 Cys Ala Gly Lys Ala Lys Arg Ser Gly Gly His Ala Pro Arg Val Arg
 245 250 255
 Glu Leu Leu Leu Asp Ala Leu Ser Pro Glu Gln Leu Val Leu Thr Leu
 260 265 270
 Leu Glu Ala Glu Pro Pro His Val Leu Ile Ser Arg Pro Ser Ala Pro
 275 280 285
 Phe Thr Glu Ala Ser Met Met Met Ser Leu Thr Lys Leu Ala Asp Lys
 290 295 300
 Glu Leu Val His Met Ile Ser Trp Ala Lys Lys Ile Pro Gly Phe Val
 305 310 315 320
 Glu Leu Ser Leu Phe Asp Gln Val Arg Leu Leu Glu Ser Cys Trp Met
 325 330 335
 Glu Val Leu Met Met Gly Leu Met Trp Arg Ser Ile Asp His Pro Gly
 340 345 350
 Lys Leu Ile Phe Ala Pro Asp Leu Val Leu Asp Arg Asp Glu Gly Lys
 355 360 365
 Cys Val Glu Gly Ile Leu Glu Ile Phe Asp Met Leu Leu Ala Thr Thr
 370 375 380

4/7

Ser Arg Phe Arg Glu Leu Lys Leu Gln His Lys Glu Tyr Leu Cys Val
 385 390 395 400
 Lys Ala Met Ile Leu Leu Asn Ser Ser Met Tyr Pro Leu Val Thr Ala
 405 410 415
 Thr Gln Asp Ala Asp Ser Ser Arg Lys Leu Ala His Leu Leu Asn Ala
 420 425 430
 Val Thr Asp Ala Leu Val Trp Val Ile Ala Lys Ser Gly Ile Ser Ser
 435 440 445
 Gln Gln Gln Ser Met Arg Leu Ala Asn Leu Leu Met Leu Leu Ser His
 450 455 460
 Val Arg His Ala Ser Asn Lys Gly Met Glu His Leu Leu Asn Met Lys
 465 470 475 480
 Cys Lys Asn Val Val Pro Val Tyr Asp Leu Leu Leu Glu Met Leu Asn
 485 490 495
 Ala His Val Leu Arg Gly Cys Lys Ser Ser Ile Thr Gly Ser Glu Cys
 500 505 510
 Ser Pro Ala Glu Asp Ser Lys Ser Lys Glu Gly Ser Gln Asn Pro Gln
 515 520 525
 Ser Gln
 530

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Thr Phe Tyr Ser Pro Ala Val Met Asn Tyr Ser
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Gly Tyr Ser Pro Ala Val Met Asn Tyr Ser
 1 5 10

5/7

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 45 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GGAAAGCTTGT CGACCACATCAT GACCGGCTAT AGCCCTGCTG TGATG

45

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGATCTAGAG TCGACGCGTC ACTGAGACTG AGGGTTCTGG

40

(2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met Thr Gly Tyr
1

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

6/7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CCATCATGAC CGGCTAT

17

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CTGGTAAGTT TAGTCTTTT GTC

23

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GCTTCACACC AAGGACTCTT TTGAG

25

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GTTGGCCACA ACACATTG TG GCTTG

26

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

7/7

(ii) MOLECULE TYPE: DNA (genomic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CCATCCTAAT ACGACTCACT ATAGGGC

27

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ACTCACTATA GGGCTCGAGC GGC

23

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 98/14944

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/12 C07K14/705 C07K16/28 G01N33/68

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	EP 0 798 378 A (AKZO NOBEL NV) 1 October 1997 see the whole document ---	1-23
X	WO 97 09348 A (KAROBIO AB ; KUIPER GEORGE G J M (SE); ENMARK EVA (SE); GUSTAFSSON) 13 March 1997 see the whole document ---	1-17, 19-23
X	MOSSELMAN S ET AL: "ERBETA: IDENTIFICATION AND CHARACTERIZATION OF A NOVEL HUMAN ESTROGEN RECEPTOR" FEBS LETTERS, vol. 392, no. 1, 19 August 1996, pages 49-53, XP002044410 cited in the application see the whole document ---	1-17, 19-23



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

30 September 1998

Date of mailing of the international search report

07/10/1998

Name and mailing address of the ISA
 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Nauche, S

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat'l Application No

PCT/US 98/14944

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
EP 0798378 A	01-10-1997	AU	1652197 A		02-10-1997
		CA	2200423 A		26-09-1997
		HU	9700636 A		28-04-1998
		JP	10004986 A		13-01-1998
WO 9709348 A	13-03-1997	AU	6988096 A		27-03-1997
		CA	2201098 A		13-03-1997
		DE	792292 T		03-09-1998
		EP	0792292 A		03-09-1997
		GR	98300029 T		30-04-1998